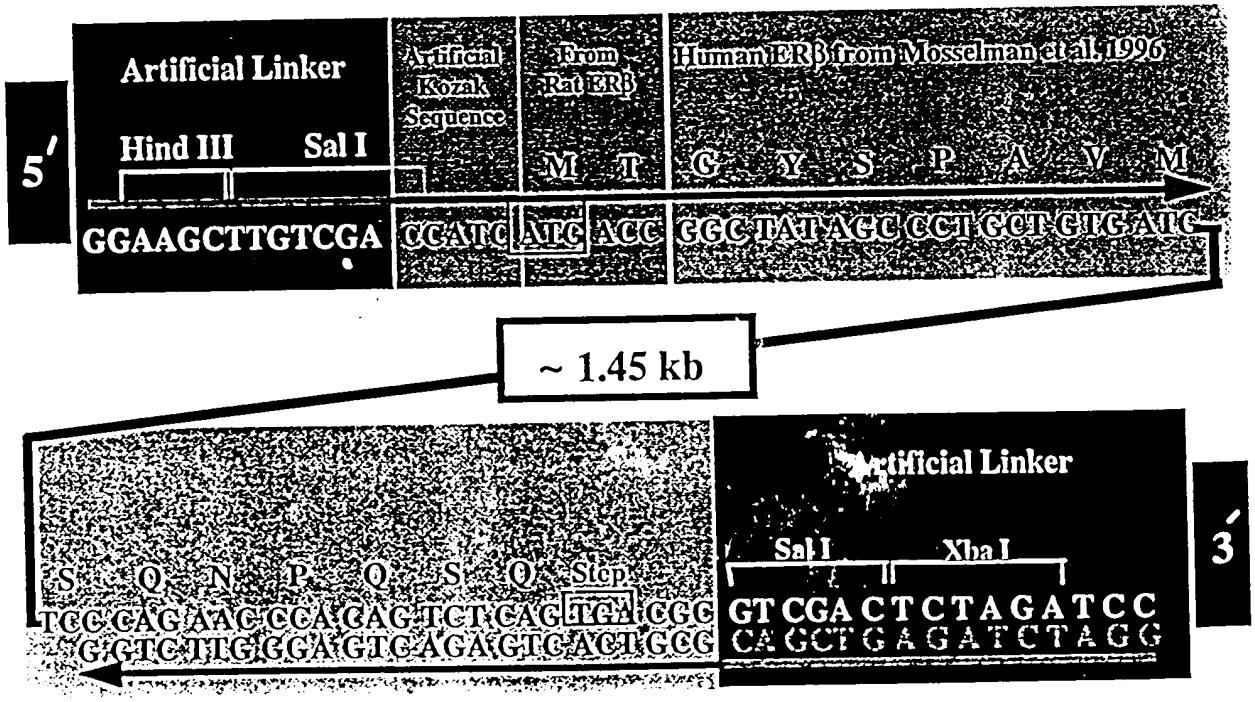
FIGURE 1

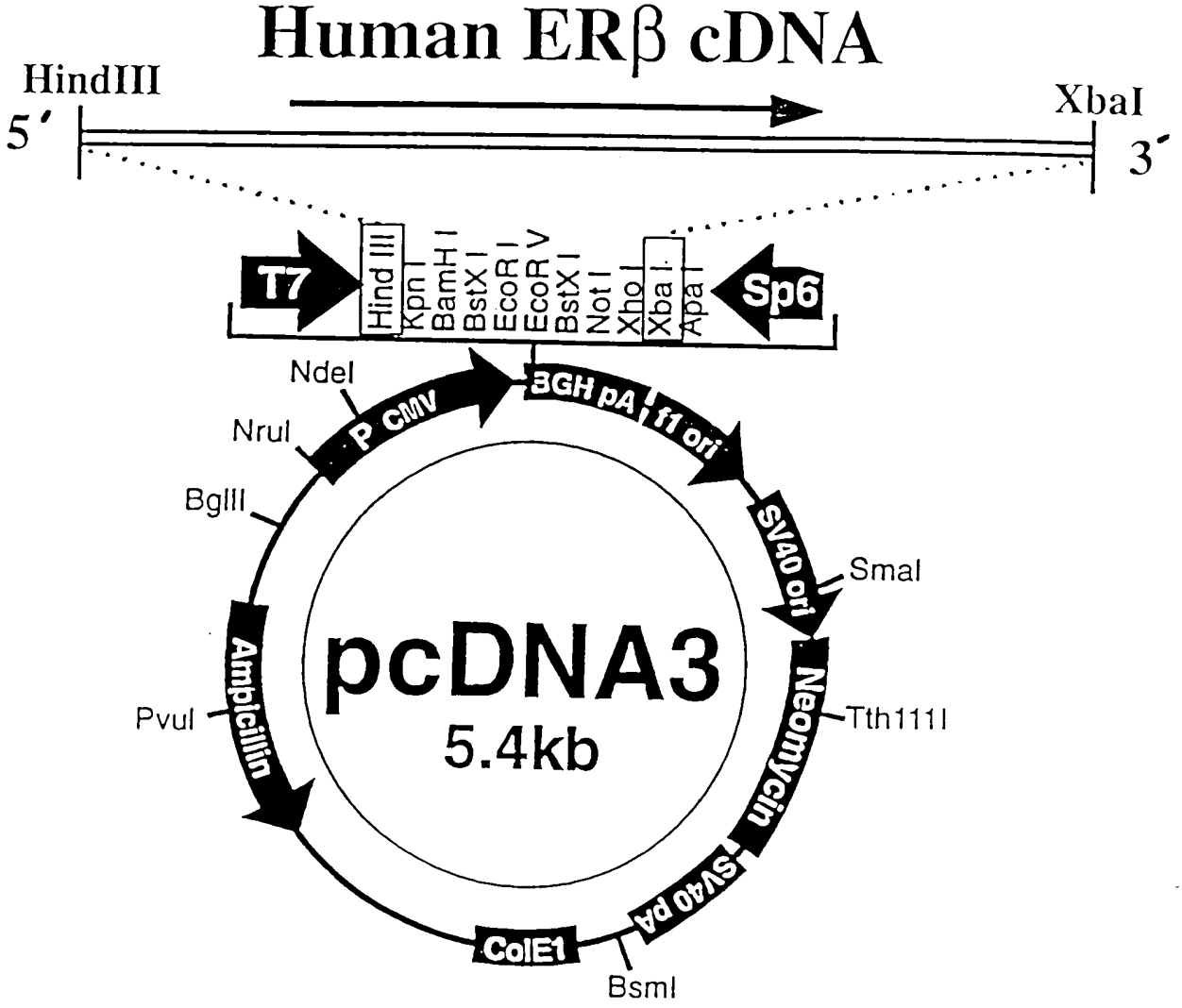
PCR Cloning of the Human ERβ

Human testis RNA was reverse transcribed using Oligo dT. The resulted transcript was used for PCR with Oligonucleotides (red arrows) designed as follows:



The PCR product was cloned in the Hind III andXbaI sites of the Eukaryotic expression vector pcDNA III.

FIGURE 2


Human ERβ cDNA
HindIII
5′
XbaI
3′
T7
Hind III
Kpn I
BamH I
BstX I
EcoR I
EcoR V
BstX I
Not I
Xho I
Xba I
Apa I
Sp6
NdeI
NruI
BglII
P CMV
BGH pA
f1 ori
SV40 ori
SmaI
pcDNA3
5.4kb
Ampicillin
PvuI
Neomycin
Tth111I
SV40 PA
ColE1
BsmI

A62

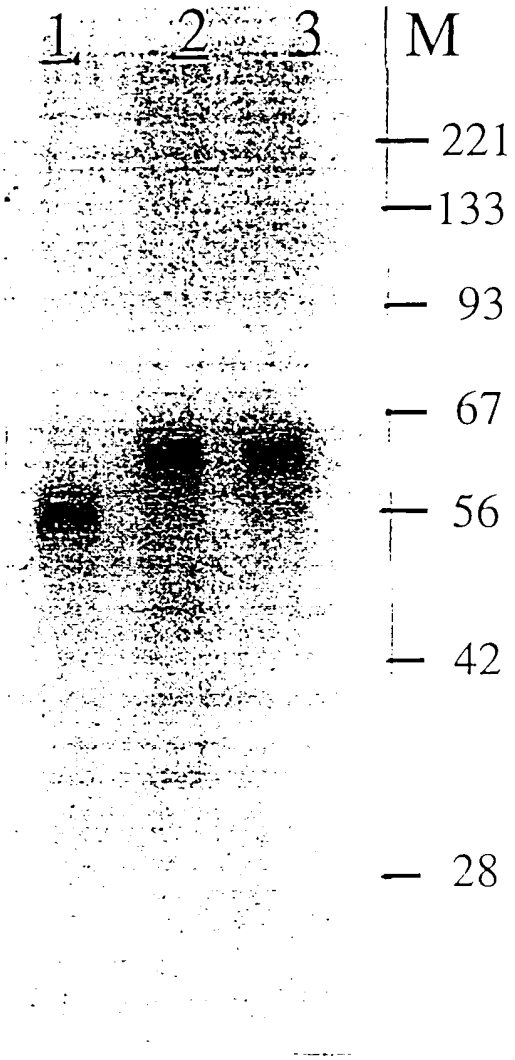
CAGCCATTATACTTGCCCACGAATCTTTGAGAACATTATAATGACCTTTGTGCCTCTTCT 60
TGCAAGGTGTTTTCTCAGCTGCTATCTCAAGACATGGATATAAAAAACTCACCATCTAGC 120
CTTAATTCTCCTTCCTCCTACAACTGCAGTCAATCCATCTTACCCCTGGAGCACGGCTCC 180
ATATACATACCTTCCTCCTATGTAGACAGCCACCATGAATATCCAGCCATGACATTCTAT 240
AGCCCTGCTGTGATGAATTACAGCATTCCCAGCAATGTCACTAACTTGGAAGGTGGGCCT 300
GGTCGGCAGACCACAAGCCCAAATGTGTTGTGGCCAACACCTGGGCACCTTTCTCCTTTA 360
GTGGTCCATCGCCAGTTATCACATCTGTATGCGGAACCTCAAAAGAGTCCCTGGTGTGAA 420
GCAAGATCGCTAGAACACACCTTACCTGTAAACAGAGAGACACTGAAAAGGAAGGTTAGT 480
GGGAACCGTTGCGCCAGCCCTGTTACTGGTCCAGGTTCAAAGAGGGATGCTCACTTCTGC 540
GCTGTCTGCAGCGATTACGCATCGGGATATCACTATGGAGTCTGGTCGTGTGAAGGATGT 600
AAGGCCTTTTTTAAAAGAAGCATTCAAGGACATAATGATTATATTTGTCCAGCTACAAAT 660
CAGTGTACAATCGATAAAAACCGGCGCAAGAGCTGCCAGGCCTGCCGACTTCGGAAGTGT 720
TACGAAGTGGGAATGGTGAAGTGTGGCTCCCGGAGAGAGAGATGTGGGTACCGCCTTGTG 780
CGGAGACAGAGAAGTGCCGACGAGCAGCTGCACTGTGCCGGCAAGGCCAAGAGAAGTGGC 840
GGCCACGCGCCCCGAGTGCGGGAGCTGCTGCTGGACGCCCTGAGCCCCGAGCAGCTAGTG 900
CTCACCCTCCTGGAGGCTGAGCCGCCCCATGTGCTGATCAGCCGCCCCAGTGCGCCCTTC 960
ACCGAGGCCTCCATGATGATGTCCCTGACCAAGTTGGCCGACAAGGAGTTGGTACACATG 1020
ATCAGCTGGGCCAAGAAGATTCCCGGCTTTGTGGAGCTCAGCCTGTTCGACCAAGTGCGG 1080
CTCTTGGAGAGCTGTTGGATGGAGGTGTTAATGATGGGGCTGATGTGGCGCTCAATTGAC 1140
CACCCCGGCAAGCTCATCTTTGCTCCAGATCTTGTTCTGGACAGGGATGAGGGGAAATGC 1200
GTAGAAGGAATTCTGGAAATCTTTGACATGCTCCTGGCAACTACTTCAAGGTTTCGAGAG 1260
TTAAAACTCCAACACAAAGAATATCTCTGTGTCAAGGCCATGATCCTGCTCAATTCCAGT 1320
ATGTACCCTCTGGTCACAGCGACCCAGGATGCTGACAGCAGCCGGAAGCTGGCTCACTTG 1380
CTGAACGCCGTGACCGATGCTTTGGTTTGGGTGATTGCCAAGAGCGGCATCTCCTCCCAG 1440
CAGCAATCCATGCGCCTGGCTAACCTCCTGATGCTCCTGTCCCACGTCAGGCATGCGAGT 1500
AACAAGGGCATGGAACATCTGCTCAACATGAAGTGCAAAAATGTGGTCCCAGTGTATGAC 1560
CTGCTGCTGGAGATGCTGAATGCCCACGTGCTTCGCGGGTGCAAGTCCTCCATCACGGGG 1620
TCCGAGTGCAGCCCGGCAGAGGACAGTAAAAGCAAAGAGGGCTCCCAGAACCCACAGTCT 1680
CAGTGA 1686

Fig 3

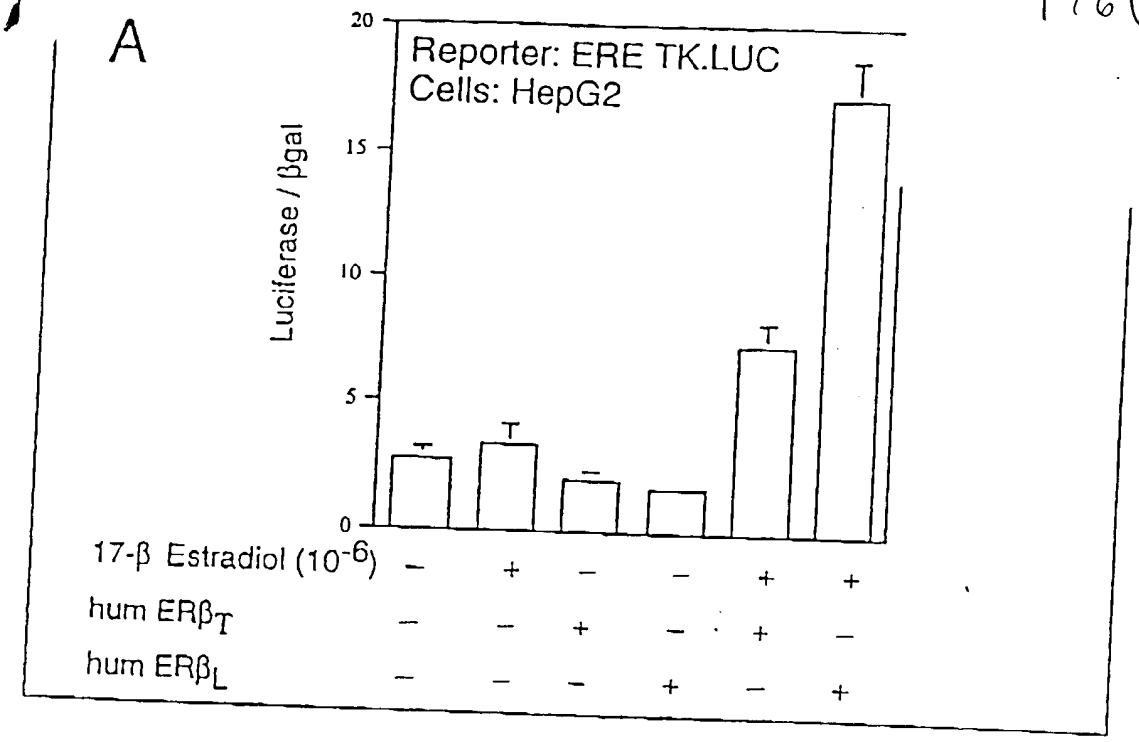
MDIKNSPSSL NSPSSYNCSQ SILPLEHGSI YIPSSYVDSH HEYPAMTFYS 50
PAVMNYSIPS NVTNLEGGPG RQTTSPNVLW PTPGHLSPLV VHRQLSHLYA 100
EPQKSPWCEA RSLEHTLPVN RETLKRKVSG NRCASPVTGP GSKRDAHFCA 150
VCSDYASGYH YGVWSCEGCK AFFKRSIQGH NDYICPATNQ CTIDKNRRKS 200
CQACRLRKCY EVGMVKCGSR RERCGYRLVR RQRSADEQLH CAGKAKRSGG 250
HAPRVRELLL DALSPEQLVL TLLEAEPPHV LISRPSAPFT EASMMMSLTK 300
LADKELVHMI SWAKKIPGFV ELSLFDQVRL LESCWMEVLM MGLMWRSIDH 350
PGKLIFAPDL VLDRDEGKCV EGILEIFDML LATTSRFREL KLQHKEYLCV 400
KAMILLNSSM YPLVTATQDA DSSRKLAHLL NAVTDALVWV IAKSGISSQQ 450
QSMRLANLLM LLSHVRHASN KGMEHLLNMK CKNVVPVYDL LLEMLNAHVL 500
RGCKSSITGS ECSPAEDSKS KEGSQNPQSQ . 531

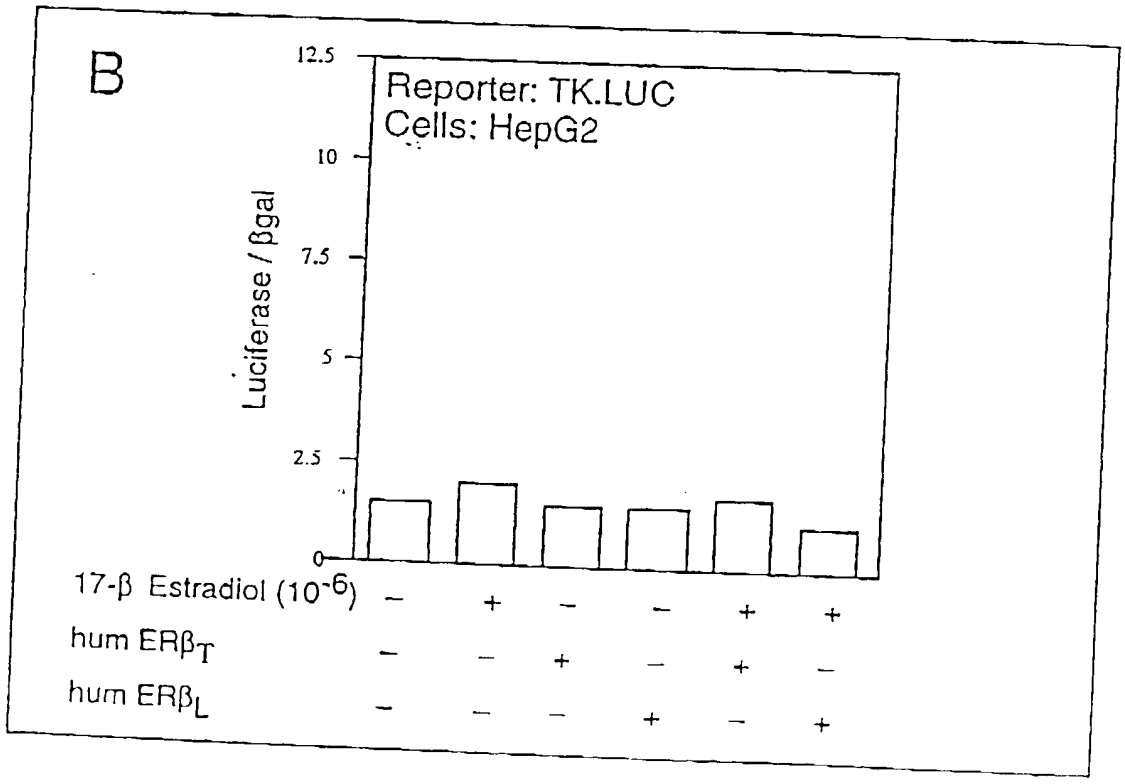
764

Fig 5

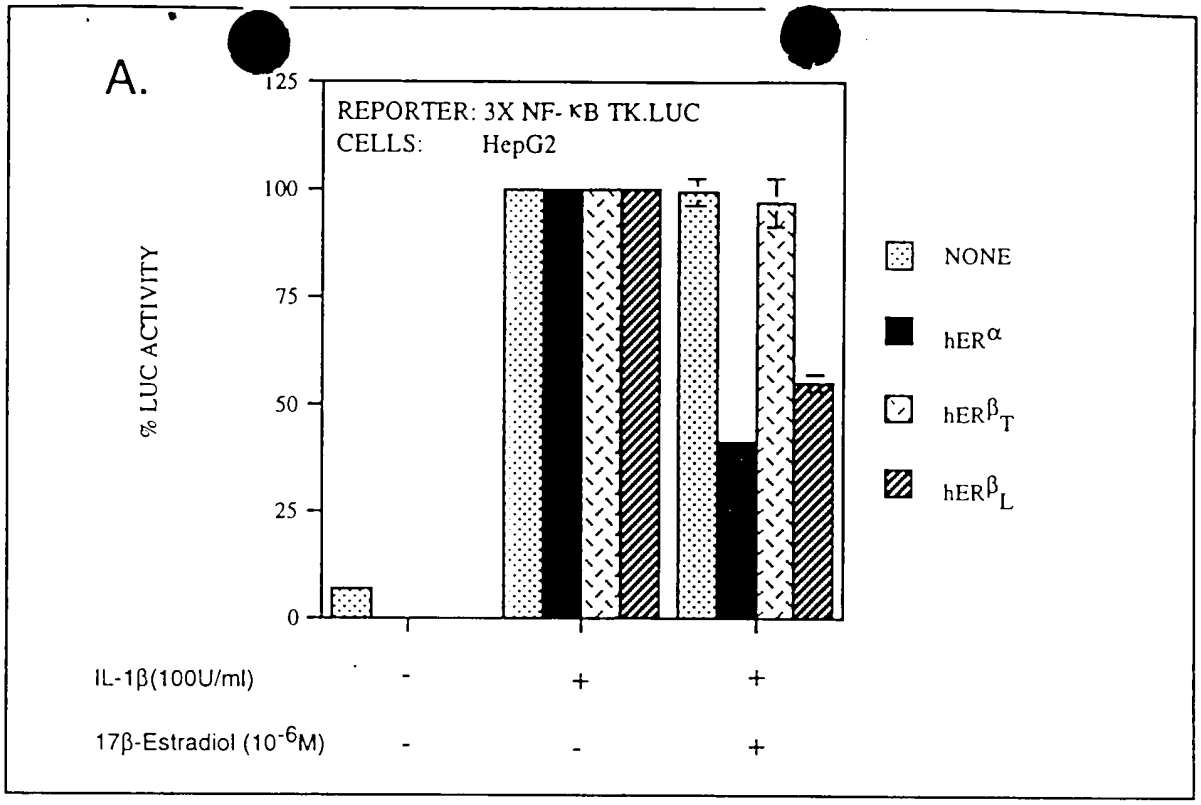


F166





Transactivation of ERE reporter By $ER\beta_L$ and $ER\beta_T$ In HepG2 cells. Luciferase reporter constructs (0.5 ug) containing either [A] the estrogen receptor DNA response element upstream of the TK basal promoter (ERE TK.LUC) or [B] the TK basal promoter alone (TK.LUC) were transiently transfected into HepG2 cells by the calcium phosphate coprecipitation method. Each construct was cotransfected with the ER expression vector (0.25 ug) indicated and the RSV-β-galactosidase plasmid (0.5 ug) to correct for variation in DNA uptake. Luciferase activity was normalized to β-galactosidase enzymatic activity.



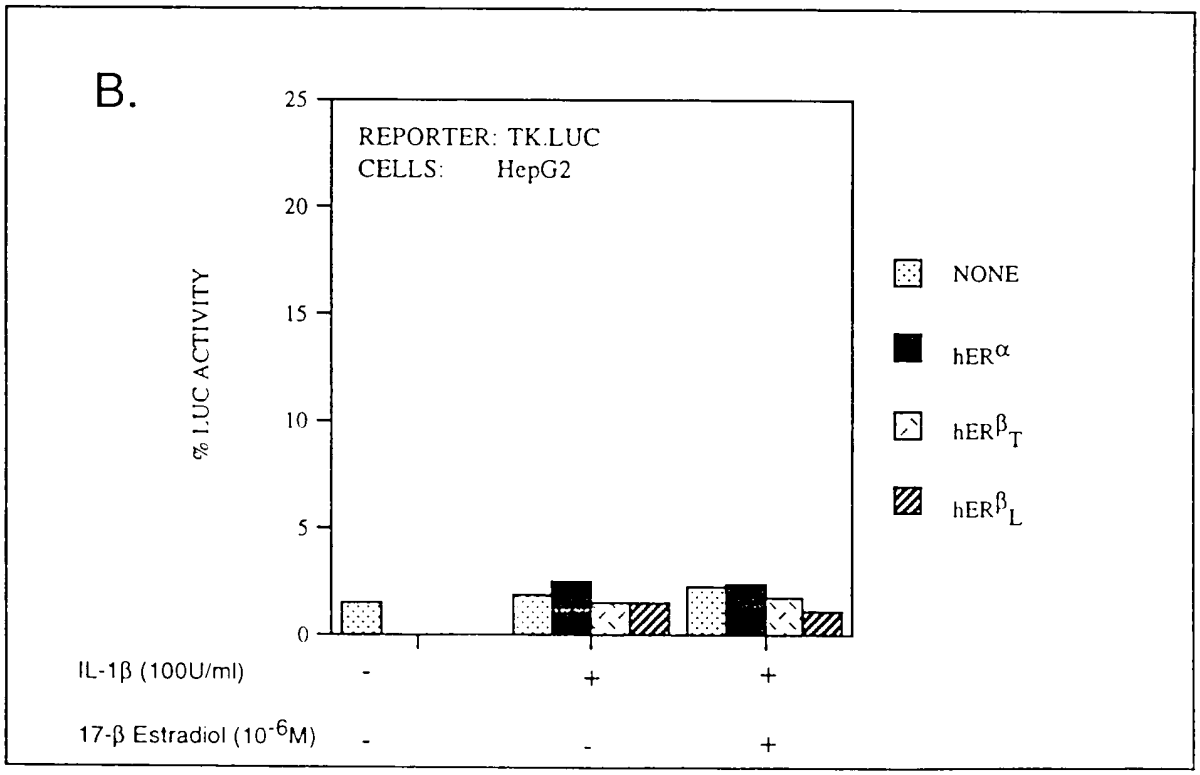
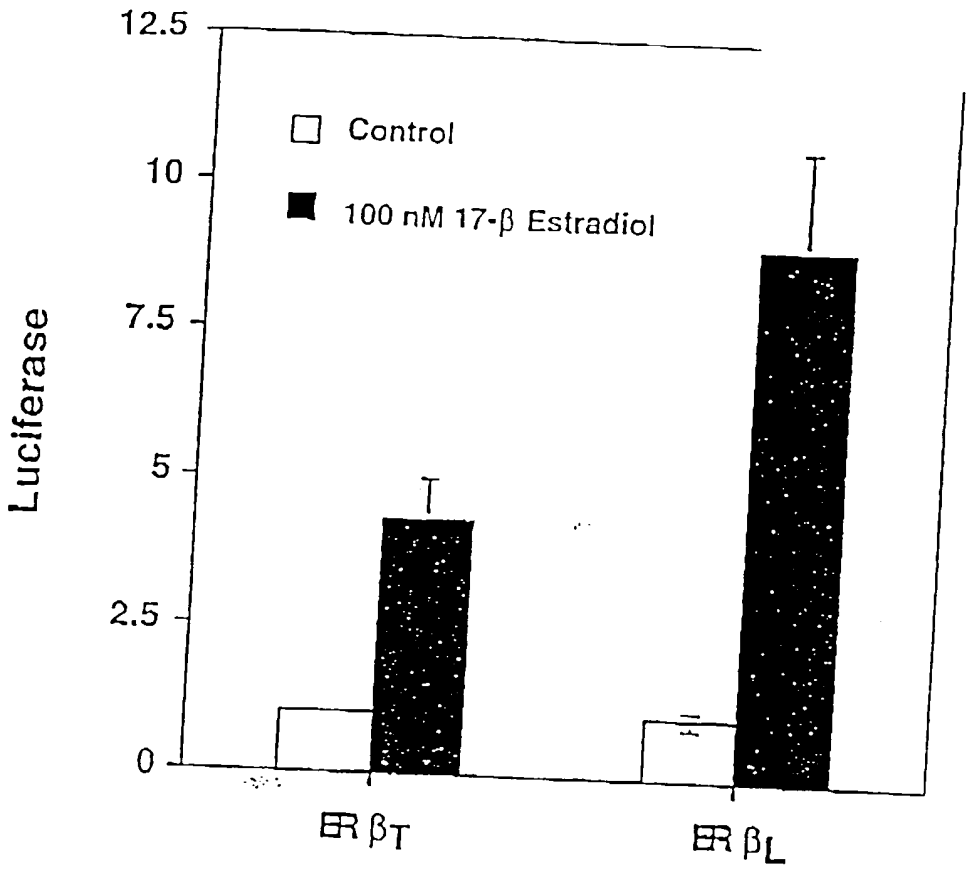


Fig 7 Luciferase reporter constructs (0.5 ug) containing either [**A**] 3 copies of an NFκB binding site upstream of the TK basal promoter (3X-NFκB TK.LUC) or [**B**] the TK basal promoter construct alone (TK.LUC) were transiently cotransfected into HepG2 cells by the calcium phosphate coprecipitation method. Each construct was cotransfected with the ER expression vector indicated and the plasmid RSV-β-galactosidase (0.5 ug) to correct for variation in DNA uptake. Percent luciferase activity values represent Luc:β-galactosidase enzymatic activity ratios relative to a value of 100% designated for the IL-1β treated samples and are presented as mean ± S.E..



Transactivation of ERE reporter by $ER\beta_T$ and $ER\beta_L$ In HAECT-1 cells. Luciferase reporter constructs (20 µg) conatining either the estrogen receptor DNA response element upstream of the TK basal promoter (ERE TK.LUC) or the TK basal promoter (TK.Luc) were transiently transfected into HAECT-1 cells ($4x10^6$) with 5 µg of ER expression vector by electroporation. Cells were plated into 48 wells of a 96-well plate, rested for 4h, and treated overnight as indicated prior to luciferase determination. ERE TK.LUC values were normalized to TK.LUC values and are presented as mean± S.E. (n=4).

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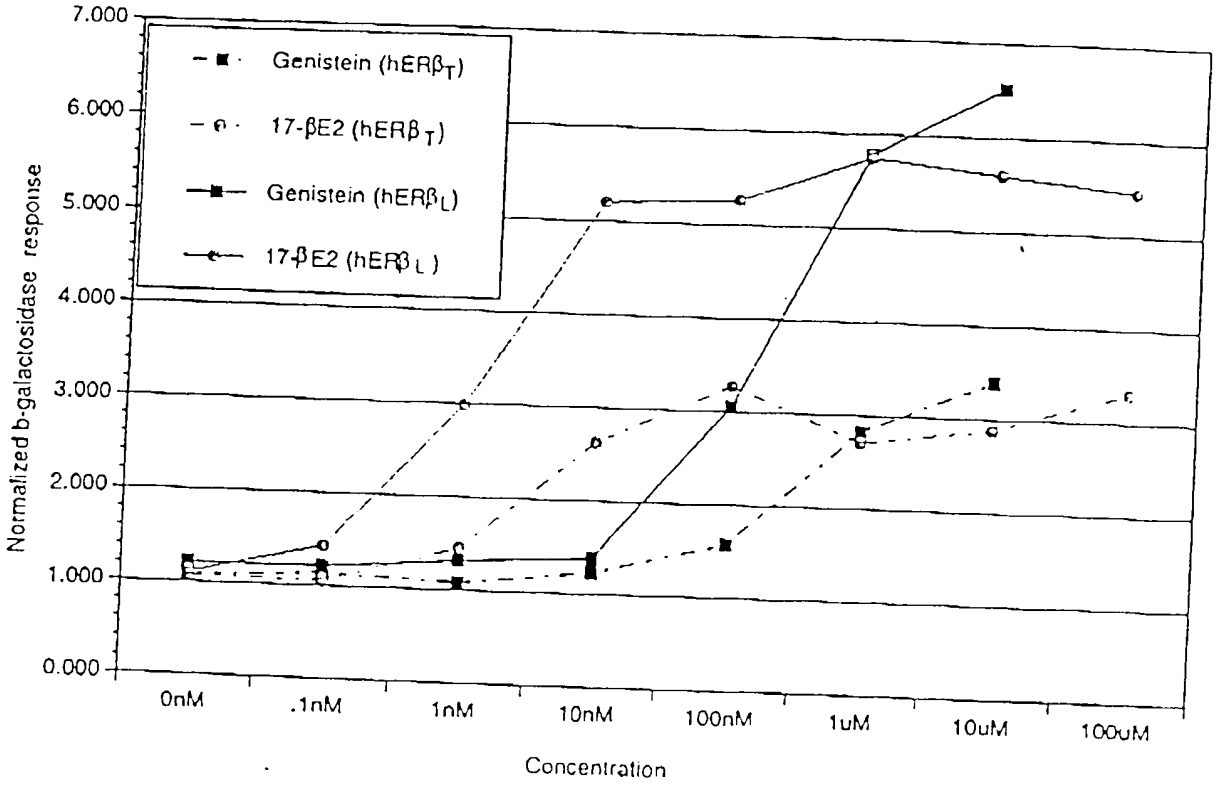

Transcriptional activity of hERβT and hERβL in yeast. Yeast cells (BJ2168) were cotransformed with an ERE-LacZ reporter (YRpE2) and either a yeast vector (pYX242) expressing hERβT or hERβL. Transformed cells were grown in selective medium for 24 h at 30oC. Cells were treated with 17-β estradiol or genestein, at the indicated concentrations, for 3 h and then assayed for β-galactosidase activity.